BACTERIAL POLLUTION INDICATORS IN HAWAIIAN COASTAL WATERS

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The use of the coliform bacteria as the indicator organism to identify fecal contamination has universal acceptance in the fields of public health and pollution control. However, it is well documented that the interpretation of the results using the coliform groups is subject to discussion particularly in relation to the marine environment (Gallagher and Spino, 1968; Orlob, 1956; ORSANCO, 1971; Savage and Hanes, 1971). Attempts to single out more direct indicators of fecal contamination that can be directly cultured and identified in a minimum of analytical time (Geldreich, 1965, 1966) have resulted in the selection of tests using fecal coliforms and fecal streptococci which are organisms specifically classified as being present in the intestinal tract of warm-blooded animals. Each test involves high temperature (approximately 45°C) and or 24-hour ±2 incubation period for complete identification. Geldreich (1970) indicated that the fecal coliform bacteria should be used as the baseline indicator rather than the fecal streptococcus group because of the lack of specificity of different streptococcus subgroups in defining water quality.

Some preliminary work has been documented, including the presence of such bacteria in natural waters from the field studies on Kaneohe Bay drainage basin by Young, et al., (1969) and the laboratory work by Leighton (1966). The latter report compares the survival in sea water of two bacteria, E. coli and S. faecalis, utilizing pure cultures, sea water dilutions, and specific culture media.

In the present study similar laboratory work was accomplished with fecal coliform and fecal streptococcus organisms to determine survival rates under varying conditions of salinity.

The initial test used unchlorinated effluent from the Wahiawa sewage treatment plant. Three trial runs were made. As the samples were brought in, they were plated. The initial number of organisms present in each sample was calculated prior to dilution with sea water in concentrations of 1/1, 1/100, and 1/1000. All samples were tested for total bacteria, total coliform, fecal coliform, and fecal streptococcus.

One liter volumes of mixtures of autoclaved, buffered distilled water and autoclaved sea water were seeded with ten cubic centimeters of the sewage effluent. The concentrations of sea water were: (1) Bottle 0, 100 percent; (2) Bottle 1, 90 percent; (3) Bottle 2, 80 percent; (4) Bottle 3,
60 percent; and (5) Bottle 4, 20 percent. The bottles were incubated at 27°C under a day/night light schedule. Samples were withdrawn at twelve-hour intervals over a ninety-six hour period and were plated onto 0.45μ membrane filters. The media and the types of organisms for which they were used were Total Bacteria and Total Coliform -- M Endo Broth, Fecal Coliform -- M -- FC Broth Base, and Fecal Streptococcus -- Enterococcus Agar.

In most cases, total bacteria and total coliform exhibited a decrease in number within the first twenty-four to thirty-six hours, but increased after the initial period of decline. However, total bacteria counts toward the last forty-eight hours were unreliable, due to an overgrowth which developed on the plates. Fecal Coliform showed a marked decrease in numbers, resulting in no count at the end of the ninety-six hours. Fecal Streptococcus showed a fluctuating system, sometimes tending toward a steady decline, other times exhibiting no defineable pattern at all. No colonies of any of the four types of bacteria formed in the control plates which were also prepared for the autoclaved distilled water and sea water used.

Since an overgrowth persistently developed in the total bacteria dishes, identifying tests were run on the organisms remaining in the bottles after ninety-six hours had elapsed. Samples were run through confirming tests: colonies were raised on Brilliant Green Lactose Broth, then transferred to the EMB Broth, and finally to a Nutrient Agar Slant. From the slant, microscopic smears were made, with the organisms staining Gram negative and being coccus in shape. The result of this work indicated that the organisms isolated were generally atypical.

A second trial was made utilizing pure cultures of bacteria, using the Wahiawa sewage, and the sea water obtained from Kaneohe. Total volume used was 2000-ml, with the percentage of salt water in each jug in decreasing amounts: Bottle 1, 100 percent; Bottle 2, 90 percent; Bottle 3, 80 percent; Bottle 4, 60 percent; and Bottle 5, 20 percent. The bottles with prepared dilutions were then autoclaved for twenty minutes. Two milliliters each of pure strains of E. coli and Streptococcus were added. Initial counts were 355 x 10^8 org/100 milliliters for E. coli and 119.5 x 10^8 org/100 milliliters for Streptococcus. These bottles were incubated at 27°C and every twelve hours samples were withdrawn and plated out. A total
of eight samples obtained at 12-hour intervals from inoculation to 96 hours were analyzed.

In this run of the experiment, because of the lack of membrane filters the procedure was changed to the pour plate method. One milliliter or one-tenth milliliter of the solution, depending upon the dilution, was pipetted onto the bottom of a large petri dish (DISPO). Ten milliliters of agar (EMB for E. coli and Enterococcus for Streptococcus) heated to 42°C were then added. The mixture was swirled in order to evenly cover the bottom. They were then incubated at 37°C for twenty-four hours for the E. coli, and forty-eight hours for the Streptococcus. All dilutions were plated in duplicate. At the end of the incubation times, the plates were counted (the graphs in Set A illustrate the results).

The results showed much variation with unusual growth and fluctuation in numbers. It was considered due to the preparation of the original dilutions. The sea water had not been filtered, therefore it was concluded that there were extra nutrients in the bottles allowing variations in growth.

In the third trial using the Wahiawa sewage and Kaneohe Bay sea water the sea water was filtered to eliminate any particulate extraneous nutrients which could become an added growth factor in the experiment. The autoclaved waters were measured out into 2000-ml jugs with the percentage of sea water to sewage in each jug occurring in decreasing amounts: Bottle 1, 100 percent; Bottle 2, 90 percent; Bottle 3, 80 percent; Bottle 4, 60 percent; and Bottle 5, 20 percent. Each bottle was seeded with 1 milliliter each of pure strains of E. coli and f. streptococcus. They were then incubated at 27°C for a total of ninety-six hours. Every twelve hours samples were withdrawn from each bottle, plated out on EMB agar, and seeded into azide dextrose broth tubes. Plates were done in duplicate, and the azide dextrose broth tubes (for Streptococcus) were done in groups of three. The tubes were incubated at 35°C for 48 hours. Because the growth on the plates was slow in forming, the plates were placed in boxes, covered, and allowed to further incubate at room temperature until the growth was large enough for counting.

There was no significant difference in the numbers (see graphs, Set B) of Streptococcus and coliform per bottle for the first three dilutions (100, 90, and 80 percent sea water). In bottles 4 and 5, however, a pattern can be detected. The coliforms had a peak at 60 hours. There was
no overall difference between coliform organisms at 12 hours and 96 hours. Streptococcus, however, showed a steady decline, resulting in a difference of $100 \times 10^7$ organisms.

Coliform organisms were affected most by the sea water. As the concentrations of sea water decreased, the peak growth increased.

**SUMMARY**

The results of testing to ascertain the effect of sea-water dilution on indicator bacteria and the relative response of fecal coliform and fecal streptococcus organisms was inconclusive. The work was handicapped by problems with the culture strains of organisms utilized and logistic difficulties which necessitated a change in technique from the membrane filter method to the multiple tube dilution most probable number method. Difficulty in securing laboratory assistance also limited the scope of the work finally accomplished. However, the laboratory help and materials that were obtained were also useful in supporting other research projects requiring identification and enumeration of these same indicator bacteria.

The erratic results and problems of aftergrowth amplify and support the reports of previous workers in the field on the difficulties involved in working with the presently utilized indicator organisms. It is recommended that further in situ work be promoted at sewage treatment plants and in receiving waters to assess the suitability of these and other indicator organisms, including Ps. aeruginosa, Salmonella species, or pathogenic viruses, because of the continuing significance of health hazards involved in water pollution control and water quality management.
REFERENCES


SET A
SET A. BOTTLE 1 (100 PERCENT SEA WATER).

**F. STREPTOCOCCUS**

- $1.195 \times 10^9$
- $3.55 \times 10^8$

**E. COLIFORM**

- $4.6 \times 10^7$

**Number of Organisms / 100 ML**

- $45 \times 10^7$
- $40 \times 10^7$
- $35 \times 10^7$
- $30 \times 10^7$
- $25 \times 10^7$
- $20 \times 10^7$
- $15 \times 10^7$
- $10 \times 10^7$
- $5 \times 10^7$

**Hours**

- H12
- H24
- H36
- H48
- H60
- H72
- H84
- H96
SET A. BOTTLE 2 (90 PERCENT SEA WATER).

E. COLIFORM

F. STREPTOCOCCUS
SET A. BOTTLE 3 (80 PERCENT SEA WATER).

NUMBER OF ORGANISMS / 100 ML

HOURS

E. COLIFORM

F. STREPTOCOCCUS
SET A. BOTTLE 4 (60 PERCENT SEA WATER).
SET A. BOTTLE 5 (20 PERCENT SEA WATER).

NUMBER OF ORGANISMS / 100 ML

E. COLIFORM

F. STREPTOCOCCUS

HOURS
SET B. BOTTLE 1 (100 PERCENT SEA WATER).

![Graph showing the number of organisms per 100 ml over time.]

SET B. BOTTLE 2 (90 PERCENT SEA WATER).

![Graph showing the number of organisms per 100 ml over time.]

- **E. Coliform**
- **F. Streptococcus**

HOURS: H12, H24, H36, H48, H60, H72, H84, H96
SET B. BOTTLE 3 (80 PERCENT SEA WATER).

![Graph showing the decline of E. Coliform and F. Streptococcus over hours.]

SET B. BOTTLE 4 (60 PERCENT SEA WATER).

![Graph showing the decline of E. Coliform and F. Streptococcus over hours.]

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**Legend:**
- ○ ○ E. Coliform
- □ □ F. Streptococcus
SET B. BOTTLE 5 (20 PERCENT SEA WATER).

- E. COLIFORM
- F. STREPTOCOCCUS